University of Hawaii at Manoa

Phyllosoma Culture

Procuring of Female Adult Specimens of *Panulirus Marginatus* and their transportation to the Waikiki Aquarium

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IS400

The objective of this project is to acquire pregnant female lobsters for experimentation on methods for the culture of phyllosoma larvae, under the auspices of Dr. Spencer Malecha.

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Acquisition of Pregnant Female Lobsters in Hawaiian Waters and their transportation to the Waikiki Aquarium.

Introduction

Aquaculture of *Panulirus Marginatus*

Mating, courtship, copulation , and egg bearing/hatching of Panulirus genera have been achieved in the lab and documented in various species, but perhaps most notably with *P. japonicas*. (Phillips, B.F., and J. Kittaka, 2000) Phyllosoma larvae culture is the last hurdle remaining to commercial aquaculture of spiny lobsters. Phyllosoma are the pelagic larvae of the spiny lobster (*Panulirus* and allied genera). During this stage of their lives the larvae float in the open ocean (Phillips and McWilliam, 1989). Their bodies are remarkably thin, flat , and transparent; the legs are very long. This body structure tends to result in high rates of attrition due to limb loss while in captivity (Matsuda and Takenouchi, 2007). In recent research the most important aspect of Phyllosoma rearing is feeding. Several foods such as *Aremia salina* nauplii and mussel gonads have been tested and found to be successful (Phillips, B.F., and J. Kittaka, 2000).
The Phyllosoma period lasts up to one per year and researchers have gradually increased the number of juveniles per spawn to 300 (Matsuda and Takenouchi, 2007). Methods to reduce phyllosoma attrition and/or decrease molt frequency include adjustment of the solar and lunar photo period, pelagic emulation tanks, and altering the mold period with hormones, and using y-peptides to increase appetite. To achieve successful rearing of the larvae researchers must experiment, and experimentation requires a source of larvae. Collection of pregnant female lobsters in the wild and hatching their eggs is the most time efficient and productive way to procure larvae, thus an underwater collection dive is required. Professor Malecha will be receiving the lobsters in flow through pelagic emulation tanks.

Anticipated Results and Expected Problems

The scientific permit allowed the procurement of 6 berried females, 6 non-berried, 6 males, and 40 juveniles of all four Hawaiian lobster species. This project covered the procurement of 6 berried females of the *Panulirus marginatus* species.
I expected to acquire at least one berried female. However collection and transportation seemed to be potential problems. During collection berried females often release their eggs when they suspect predation. Thus females with very immature eggs must be collected to prevent premature release. Secondly transportation may inhibit oxygen from reaching the egg sacs leading to infertile eggs on arrival. Several methods from immersion in water with air stones to wet towels in dry coolers will be tried.

Preliminary Research
The Big Island is substantially more abundant in its bio-resources than Oahu and by doing preliminary dives, around Oahu, at the start of the project, we ascertained it would be the most beneficial to procure the females on the Big Island. A trip to Molokai was considered to procure slipper lobsters, in case the procurement on Hawaii was not successful.

Supplied Resources and Materials
I was responsible operationally and financially for the capture and transportation of live, viably pregnant, female lobsters to their habitats. Spencer Malecha, the advisor, supplied the location and the habitats at the Waikiki aquarium, the scientific permit required to capture pregnant lobsters and compensation of $250 per living fertile lobster delivered to the Aquarium. Scuba Diving air fills and boat access were generously donated by Breeze Hawaii Adventures Corporation.

Expenses
Dive gear, snorkel and scuba were personally owned, and required for the project. A temporary habitat for the lobsters during transportation consisted of a Styrofoam cooler with a battery powered air stone to provide oxygen. The coolers' insulation helped prevent radical temperature shifts, which may cause premature death of the females or their eggs. These materials were purchased out of personal funds. The necessary permits for shipping pregnant female lobsters were also purchased. Personal and donated expenses are summarized below:

- Round trip airline ticket for 6 lobsters and 2 humans ~$400
- Styrofoam tanks and proper shipping permits ~$200
- Compressed air, boats and captain, and equipment ~$1000 (donated)
- Kriesel Tank ~$100

Total Costs ~$1700

Results and Accomplishments

Collection and Transportation of *Panulirus penicillatus* on Big Island
Six lobsters were collected on the Big Island and transported successfully through the airport and to the Aquaculture Laboratory holding facility. They were eventually transported to the Waikiki Aquarium. Of the six lobsters one hatched its egg clutch. The lobsters were reared to stage 2 in approximately 8 days.

Collection of *Arctides regalis* on Oahu
One berried female lobster was collected here in Oahu at horseshoe reef. Hideki Oseto collected it. The lobster was brought to the Waikiki Aquarium were it successfully

hatched its eggs. The phyllosoma are still currently growing out and have reached stage 2.

Construction of Lab Grow-out Tank

A Kreisel grow-out tank was constructed for lobster phyllosoma growout. I built it from the ground up in my living room with simple tools such as a drill and router. Garage sale fish tank filters ~$10 and leftover lab gravel filters were employed. The 10 gallon tank was procured from the roadside for 0$. The hoses, glue, and Plexiglas were purchased from home depot for ~$70 and the filter screen was bought from a t-shirt printing screen supplier for ~$20. Operation of the tank was quite successful compared to individual housing in 250ml flasks. However, the Aquarium set up out performed them both due to superior flow through design. The lab tank was recirculating and relied on simple biological filtration. A protein skimmer, UV sterilizer, and antibiotics that the Waikiki Aquarium had would have helped greatly, however funds were limited.

Rearing of Lab Phyllosoma

Phyllosoma from both species collected were placed into the Kriesel tank, the first batch of *Panulirus penicillatus* died off after 8 days. *Arctides regalis* also was raised to stage 2 and continues to survive. In addition to the Kriesel tank phyllosoma were also reared in individual containers to isolate the instar molts and timing of the molts. Feed prepared was *Artemia salina.*

Styrofoam Cooler

Upon arrival at the airport with my fancy Styrofoam container I was informed that Styrofoam is not allowed on the airlines due to strength issues. Thus once I arrived in the Big Island I purchased a real cooler from Wal-Mart. I also made a point to ask about shipping procedures for the lobster. I was informed that my cooler must be dry and the material inside to be bagged in plastic. I then informed them that plastic would kill my living lobster and negotiated their transportation successfully with the manager.

Discussion

Lobster collection was successful, as the females did not strip their eggs before arriving at the Aquaculture Laboratory holding facility. However, once in the Waikiki Aquarium most of the lobsters did not hatch their eggs due to bacterial infection of the eggs. Also preparation of the food the for the first batch was not ready when they hatched so death occurred soon after.

Evaluation of Learning

What did I learn/ gain during this project?

a) Lobster Diving- I gained more experience diving at night in adverse conditions.
b) Lobster Lifecycle- Dr. Malecha informed me that he needed lobsters for a project of his. I told him I had a source. Seeking to be on the project I flooded my brain with lobster rearing books (really only two books extant). I learned about Phyllosoma being pelagic, their migration with the currents, and how they settle in faraway places. Quite a romantic saga.
c) Kreisel Tank- The fact that the phyllosoma were pelagic intrigued me. Even though I had many years in the diving industry I had never realized how much of the ocean species were floating in the blue. So I started to observe species in the water column and began to see amazing creatures that the eye can barely see. I saw a few jellyfish in the Waikiki Aquarium and the tank that held them was circular to suspend them. This emulated the pelagic environment. I found a site that built and sold similar tanks and Dr. Malecha purchased one. I decided that the tank was too expensive so I built my own using.
d) Dry Transportation- I was informed by many that the best way to ship a lobster is with a wet towel in a empty cooler. This did not seem rational to me but I deferred to age and trusted my sources. Alas it was successful.
e) Digital Microscope Photography- It was my job to procure and master a digital microscope for the lab. I fixed the computer in the lab one day, so Dr. Malecha decided that qualified me to purchase a microscope. I had only used one for the first time a few months prior. So I immersed myself in the literature of microscopes to learn the different types and what they were used for. I scoured the internet for these various types and after several weeks I had quoted the type of scope we needed with a 5mp digital camera for ~$400. $400 dollars less than my budget. So we ordered it and I installed the camera and software. I spent another few days learning how to take decent pictures and taught others in our lab the tricks I had learned.

What did you learn about yourself?

I learned that my training as a Philosopher taught me how to solve problems by teaching myself previously unknown knowledge through research skills I gained doing essays in philosophy. Once I learned to use the library and the internet effectively for philosophy I had access to all the collected knowledge of all mankind. Being able to read critically was one of the greatest skills I acquired in Philosophy and it finally came into

practice for something practical and intellectual. For a long time I thought that my degree was useless, but MOP taught me that I can apply my skills to anything. I jumped into aquaculture and digested it and in less than a year I was on two major research projects. I found that I could keep up intellectually with other students who had years of specific training. While I may not have had the training of all marine biology I was able to get "up to speed" relatively quickly. I think that this kind of training is exactly what businesses want from a college graduate.

Acknowledgements

f) Hawaiian Airlines- for transporting the lobster.
g) Waikiki Aquarium- for housing the lobster.
h) Breeze Hawaii- for supplying dive gear, air, and boats.
i) Spencer Malecha Ph. D- for guidance and insight into lobsters.
j) Hideki Oseto- for collecting our *Arctides regalis* specimen.
k) James Santos- for having a spot to collect lobster and collecting most of them.
l) Jeff, Cindy, and MOP- for supporting and evaluating my project.

Overall Summary of Experience

Overall I feel as if I can accomplish anything that I set my self too. I've always heard that all my life, but it's nice to have a real tangible project to apply myself to rather than some idea or concept like truth and consciousness. I learnt a great deal about the ocean that I spend every day diving. It's totally transformed my dive life, I see so much more than I used to. This project and my marine training has made me a better dive guide because I know that even in the most apparently barren place in the ocean, life it teeming if you only know where to look.

References

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Reskoff, K.A., F.A. Sommer, W. H. Hammer, and K.M. Cross. “Collection and Culture Techniques for Gelatinous Zooplankton.” Biol. Bull 204 (2003): 68-80.

Phillips, B.F., and J. Kittaka, eds. “Spiny Lobsters: Fisheries and Culture.” Malden, Mass.: Fishing News Books (2000): 486-503.

Figures

Figure 1. Site of Procurement. The figure depicts the site location for pregnant female lobster collection on Hawaii Island, and the boat used, which was borrowed from Breeze Hawaii dive shop.

Figure 2. Crab Cage Holding Facility. The cage was used to hold lobsters in the ocean until they were ready to be transported to Oahu.

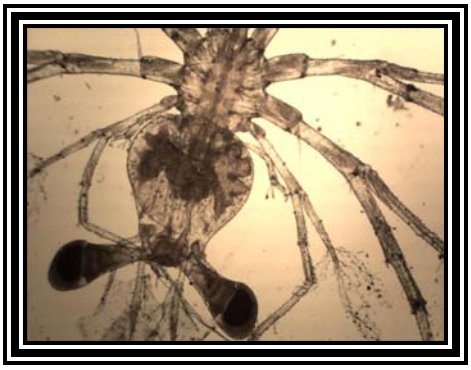

Figure 3: Photograph of a lobster phyllosoma. A photograph taken of lobster phyllosoma from a collected female lobster, under the microscope.

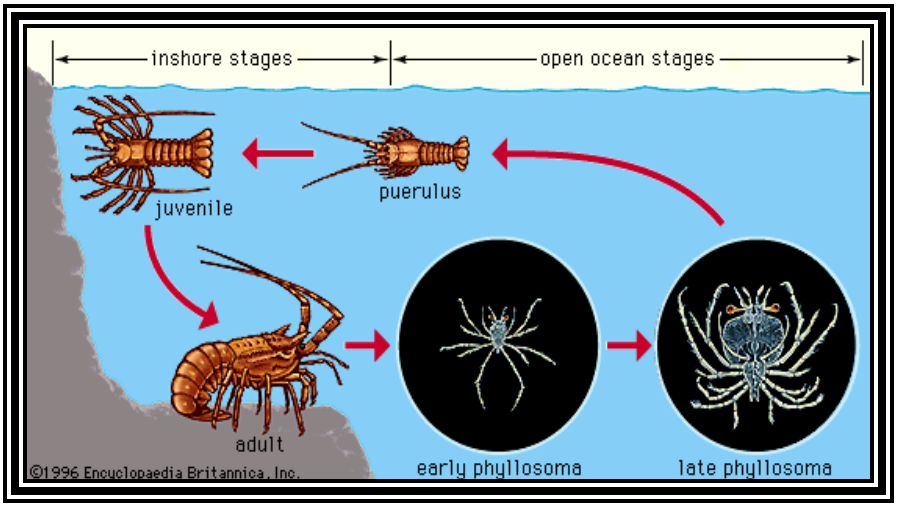

Figure 4. Lobster Life Cycle. This figure shows the stages on lobster growth in a life cycle

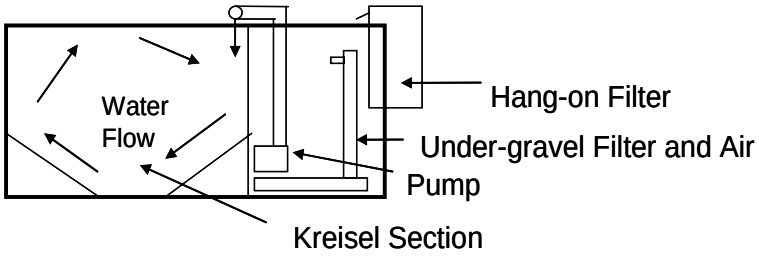

Figure 5. Kreisel Tank Diagram of Operation and Photo. Water within the tank flows in a circular motion. The design was adapted from the original kreisel tanks invested in the Montery Bay Aquarium

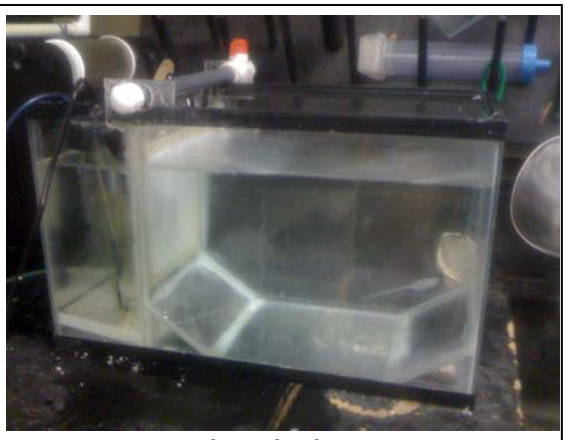

Figure 6. Kreisel Tank Photo. This figure shows the tank that I built for the lobster phyllosoma.

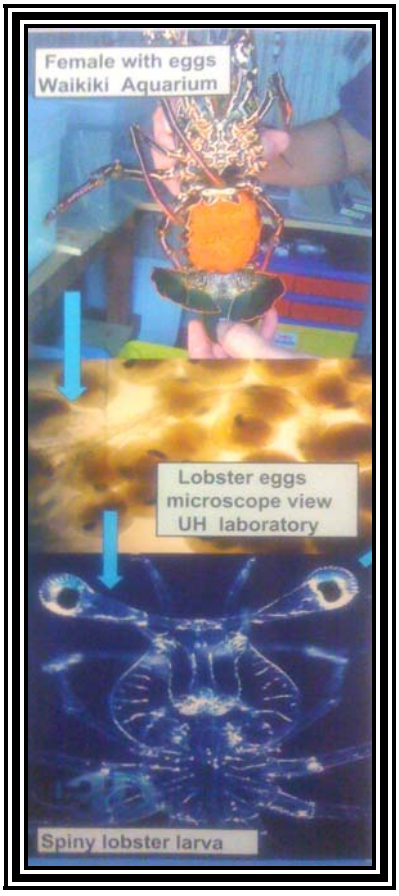

Figure 7. Female Eggs and Lobster Eggs Microscope View.